



# Developmentally-Inspired Shrink-Wrap Polymers for Mechanical Induction of Tissue Differentiation

## Citation

Hashmi, Basma, Lauren D. Zarzar, Tadanori Mammoto, Akiko Mammoto, Amanda Jiang, Joanna Aizenberg, and Donald E. Ingber. 2014. "Developmentally-Inspired Shrink-Wrap Polymers for Mechanical Induction of Tissue Differentiation." *Advanced Materials* 26 [20] (February 18): 3253–3257. doi:10.1002/adma.201304995.

## Published Version

doi:10.1002/adma.201304995

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# **Developmentally-Inspired Shrink-Wrap Polymers for Mechanical Induction of Tissue Differentiation**

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Keywords: scaffold engineering, biomaterials, odontogenesis, poly(*N*-isopropylacrylamide), thermoresponsive

Local and abrupt changes in mechanical forces play a fundamental role in control of tissue and organ development. While some investigators have varied material properties of tissue engineering scaffolds to influence cell behavior, no biomaterials have been developed that harness mechanical actuation mechanisms to induce new tissue formation. Here, we describe the development of mechanically-actuatable polymers that induce tissue differentiation by harnessing the physical induction mechanism that drives tooth organ formation in the embryo. The formation of many epithelial organs is triggered when sparsely distributed mesenchymal cells abruptly pack closely together and undergo a “mesenchymal condensation” response. For example, in tooth development, the associated physical compression and rounding of dental mesenchymal cells is sufficient to induce whole organ formation *in vitro* and *in vivo*.<sup>[1]</sup> Inspired by this developmental induction mechanism, we fabricated an artificial, shrink-wrap like polymer scaffold that can stimulate tooth tissue differentiation by abruptly inducing physical compaction of cells cultured within it when warmed to body temperature. A porous, GRGDS-modified, hydrogel scaffold was fabricated from poly(*N*-isopropylacrylamide) (PNIPAAm), which remains in an expanded form in the cold, and rapidly contracts volumetrically when placed at body temperature. When undifferentiated embryonic dental mesenchymal cells were seeded within this hydrogel sponge and polymer shrinkage was thermally induced by warming, the cells became physically compressed and exhibited a more compact, rounded morphology, as they do when they undergo mesenchymal condensation during tooth organ development in the embryo. This physical change in cell shape stimulated tooth differentiation, as measured by the induction of key odontogenic transcription factors *in vitro* and stimulation of mineralization *in vivo*. This polymer-based mechanical actuation mechanism represents a new bioinspired approach to induce organ-specific tissue differentiation that could be useful for stem cell biology, tissue engineering and regenerative medicine.

Current design strategies used to fabricate materials for tissue engineering and regenerative medicine focus on the chemistry, structural properties, and three-dimensional (3D) spatial organization of the components that comprise these scaffolds (e.g., polymers, ceramics, biomaterials).<sup>[2,3]</sup> While current tissue scaffold designs can support cell survival and maintenance of some differentiated cell functions, they do not exhibit the ability to induce major developmental lineage switches that can drive whole organ formation. Thus, we set out to develop materials that mimic the organ inductive properties of certain embryonic tissues. The formation of most organs in the embryo results from complex interactions between adjacent epithelial and mesenchymal tissues.<sup>[4-7]</sup> An initial instructive signal, provided by one of the tissue layers, is followed by reciprocal exchange of inductive signals, resulting in stepwise differentiation of both tissue components into an integrated organ structure. One of the simplest examples of organ formation is the development of the tooth. In the mouse, the embryonic day (E10) dental epithelium induces a ‘mesenchymal condensation’ response in which underlying mesenchymal cells are stimulated to migrate towards the base of the epithelium, resulting in formation of a tightly packed, dense cell aggregate in this region.<sup>[1,8,9]</sup> Recent studies have revealed that the physical compression of cells caused by this condensation response mechanically triggers the mesenchymal cells to express odontogenic (tooth forming) genes including Pax9, Msx1 and BMP4.<sup>[1,10,11]</sup> Moreover, once induced in this manner, the mesenchyme can support whole tooth formation when recombined with normal embryonic dental epithelium and implanted under the kidney capsule in a mouse.<sup>[1]</sup> Importantly, similar mesenchymal condensation processes are crucial for the formation of many other epithelial organs, including the salivary gland, pancreas, kidney, bone, and cartilage among others<sup>[8,12-16]</sup>, and thus, harnessing this induction mechanism could have much broader value for the field of tissue engineering.

Inspired by this mechanical organ induction mechanism, we set out to explore whether it is possible to develop artificial polymer scaffolds that can abruptly shrink in 3D, and



thereby physically compress mesenchymal cells cultured within the lattice to induce their

differentiation. We designed a temperature-responsive polymer scaffold composed of poly(*N*-isopropylacrylamide) (PNIPAAm), which upon heating to physiological temperatures contracts in volume in 3D. PNIPAAm has been previously shown to autonomously change volume through alteration of temperature within physiologically relevant ranges.<sup>[17-20]</sup> The thermosensitivity of PNIPAAm is governed by its lower critical solution temperature (LCST): at temperatures below the LCST, the polymer is swollen, whereas it contracts when the polymer is heated to higher temperatures.<sup>[21,22]</sup> PNIPAAm's LCST also can be manipulated by adding other chemical moieties that change the hydrophilicity of the polymer, thereby allowing its thermoresponsiveness to be tailored for specific biological applications.<sup>[17, 19, 22]</sup> Other parameters such as volume change, porosity, and biocompatibility also can be controlled by altering cross-linking density or adding adhesion-promoting components.

In the present study, we incorporated a GRGDS peptide into the cross-linked PNIPAAm polymer gel that mediates adhesion to cell surface integrin receptors in order to promote cell anchorage and survival.<sup>[23-25]</sup> The peptide was first modified with an acrylate moiety so that it could be polymerizable and then mixed into the hydrogel precursor solution containing 10% *N*-isopropylacrylamide (NIPAAm) monomer and 1% *N,N'*-methylenebisacrylamide (BIS) cross-linker by weight in water (**Figure 1a**). The hydrogel was polymerized by radical initiation using ammonium persulfate (APS) and tetramethylethylenediamine (TEMED), and then subsequently lyophilized (see Supporting Information for details). While PNIPAAm without peptide has an LCST of 32°C, the addition of even low concentrations of GRGDS significantly increased the LCST of the gel. For this reason, we experimentally determined the optimal concentration of peptide monomer necessary to yield hydrogels with an LCST of ~36°C, which is close to body temperature (**Supplementary Figure S1**). Swollen gels without cells were found to have an average pore size of 2398 ± 211 μm and 1618 ± 108 μm in their contracted state as determined from

contracted volumetrically by approximately 45% when heated from 34°C to 37°C in medium containing 10% fetal bovine serum (**Figure 1b-c**). The volumetric response was also reversible in that GRGDS-PNIPAAm gels that contracted at 37°C could be induced to swell back to their original size by lowering the temperature.

To determine whether cells cultured within the temperature-responsive scaffolds could be physically compressed by inducing gel shrinkage, dental mesenchymal cells originally isolated from E10 mouse embryos were implanted within the GRGDS-modified PNIPAAm gel, cultured overnight at 34°C, and then either maintained at the same temperature or shifted to 37°C for 48 hours. Importantly, while cells maintained at the lower temperature exhibited polygonal cell morphology (**Figure 2a**) similar to that observed on conventional culture substrates, these cells formed tightly compacted, dense aggregates and exhibited a round morphology when the the hydrogel was induced to shrink by raising the temperature to 37°C (**Fig. 2a-c**). Computerized morphometric analysis confirmed that projected cell areas reduced by more than 60% when this compression response was mechanically triggered (**Fig. 2c**), which is similar to the rounding and reduction in cell size that was shown to be sufficient to induce these dental mesenchymal cells to undergo odontogenic differentiation *in vitro* and *in vivo*.<sup>[1]</sup> Moreover, time-lapse 2D and 3D imaging of the same gel revealed that cell rounding response occurred in parallel with gel contraction and that this occurred rapidly (< 15 min) after the temperature was raised (Supplementary Videos V1 and V2, respectively), thus, confirming that this a direct effect of mechanical compaction. Although the rate of the cell shape change varies depending on how quickly the heating chamber of the microscope equilibrated to the warmer temperature, the cell volume decreased at approximately  $1.64 \times 10^5 \mu\text{m}^3/\text{min}$  in our 3D time-lapse study (Supplementary Figure S2). Importantly, most of the cells cultured at 37°C in the contracted GRGDS-gels remained viable for at least two weeks in culture as determined by cell viability, cytotoxicity, and growth assays, whereas cells on

within the first week (Supplementary Figures S3 & S4).

Next, we explored whether the cell shape change and compaction induced by the hydrogel compression influenced expression of three different genes that are critical drivers of tooth formation - Pax9, Msx1, and Bmp4.<sup>[1,26,27]</sup> Immunofluorescence staining for Pax9 protein confirmed that increased numbers of cells expressed this odontogenic transcription factor when cultured in contracted gels placed at 37°C that induced cell rounding compared to swollen gels maintained at the lower temperature where no compaction was observed (**Figure 3a**). Similar responses were observed when we used PCR to quantitate changes in mRNA levels for Pax9 as well as for two other genes – Msx1 and Bmp4 – that are also key drivers of tooth formation<sup>[1,26,27]</sup> (**Figure 3b**). Pax9 mRNA levels doubled in contracted gels relative to cells in gels maintained at 34°C, and Msx1 and Bmp4 increased by ~1.5-fold within 2 days after stimulation from hydrogel contraction ( $p<0.05$ ). Expression of these odontogenic markers was not affected by these temperature differences alone as confirmed by control experiments with cells cultured at 34°C and 37°C under standard culture conditions (**Supplementary Figure S5**).

The long-range goal of this effort is to design and fabricate biomimetic inductive scaffolds developmental induction for *in vivo* tissue engineering. To begin to explore the feasibility of this approach, GRGDS-PNIPAAm gels seeded with  $1 \times 10^6$  dental mesenchymal cells were implanted under the kidney capsule of an adult mouse using a published method for *in vivo* analysis of tooth formation (**Figure 4a & Supplementary Figure S6**).<sup>[1,28]</sup> As these gels spontaneously contract when placed at body temperature, a cell pellet containing the same number of cells without a scaffold was implanted as a control. Additional *in vivo* controls included implantation of the GRGDS-PNIPAAm gel alone without DM cells and use of a GRGDS-PNIPAAm gel designed with an LCST above 37°C containing the same number of DM cells.

Histological analysis of these implants after 2 weeks revealed that only the contracted gel containing cells implanted within the shrink wrap GRGDS-PNIPAAm polymer induced neovascularization (Figure 4a), and physical compaction of the DM cells could be detected *in vivo* (Supplementary Figure S7). Staining with Alizarin Red S and alkaline phosphatase (ALP) revealed that only the implants containing cells within contracted GRGDS-PNIPAAm gels were positive for deposition of calcium and mineralization, respectively (Figure 4b-c), which are indicative of later stages of tooth formation.<sup>[1,16,27,28]</sup> In contrast, neither mineralization nor vascularization was observed when the cell pellet or gel was implanted alone, or when the higher LCST gel (that did not contract at 37°C) with DM cells was implanted (Fig. 4b-c). Taken together, these results clearly demonstrate that mechanical compression of DM cells within the contracting gel was required for the induction of the mineralization and vascularization we observed.

These findings confirm that a developmentally-inspired biomimetic scaffold that induces mesenchymal condensation mechanically can potentially be used to therapeutically stimulate cell and tissue differentiation *in vitro* as well as *in vivo*. In past studies, we showed that physical compression of cells during the mesenchymal condensation process is the key signal that triggers tooth formation, and that this is mediated by cell shape-dependent changes in the expression of two key odontogenic transcription factors (Pax9 and Msx1) and one important morphogen (Bmp4).<sup>[1]</sup> The results of the present study confirm that physical compaction of dental mesenchymal cells is indeed the key regulator of this tooth differentiation pathway. Responsive polymers have been previously used for controlled release of drugs and cells<sup>[19,29]</sup>, and PNIPAAm has been employed to control cell adhesion and release tissues from substrates after they have formed.<sup>[30]</sup> But to our knowledge, this is the first study demonstrating the use of a responsive polymer, such as PNIPAAm, to induce tissue differentiation specifically by mechanically actuating a cell compaction response. It is also

response.

We only focused on the effects of polymer shrinkage-induced compression of dental mesenchymal cells on tissue differentiation in the present study because inclusion of dental epithelial cells would have complicated our analysis. However, previous work has shown that induced dental mesenchymal cells must be recombined with dental epithelial cells in order to produce fully formed teeth *in vivo*.<sup>[1,28]</sup> Thus, tissue recombination studies should be explored in the future to fully define the value of this approach for organ engineering applications.

Many other organs require mesenchymal condensation for their induction and formation, including salivary gland, pancreas, kidney, bone, and cartilage,<sup>[12-16]</sup> and so these inductive polymer gels could have value for engineering of many tissues. Mechanically actuating polymer systems potentially could be used to suppress cancer growth as past studies have shown that tumor expansion can be accelerated or suppressed by altering tissue mechanics and cell distortion.<sup>[31,32]</sup> Thus, this shrink wrap polymer strategy will likely have broad applications for tissue engineering, regenerative medicine, and clinical therapy as well as value for basic research aimed at understanding and manipulating organ formation and regeneration.

## Experimental

Experimental materials and methods are provided in the Supporting Information.

## Acknowledgements

This work was conducted with support by grants from the NIH Common Fund (RL1DE019023 to D.E.I.), the Wyss Institute for Biologically Inspired Engineering at Harvard University, and partially from the DOE BES (DE-SC0005247 to J.A.). We would like to thank E. Jiang and M. Kowalski for their technical assistance and T. Ferrante for assistance in imaging.

Received: ((will be filled in by the editorial staff))

Revised: ((will be filled in by the editorial staff))

Published online: ((will be filled in by the editorial staff))

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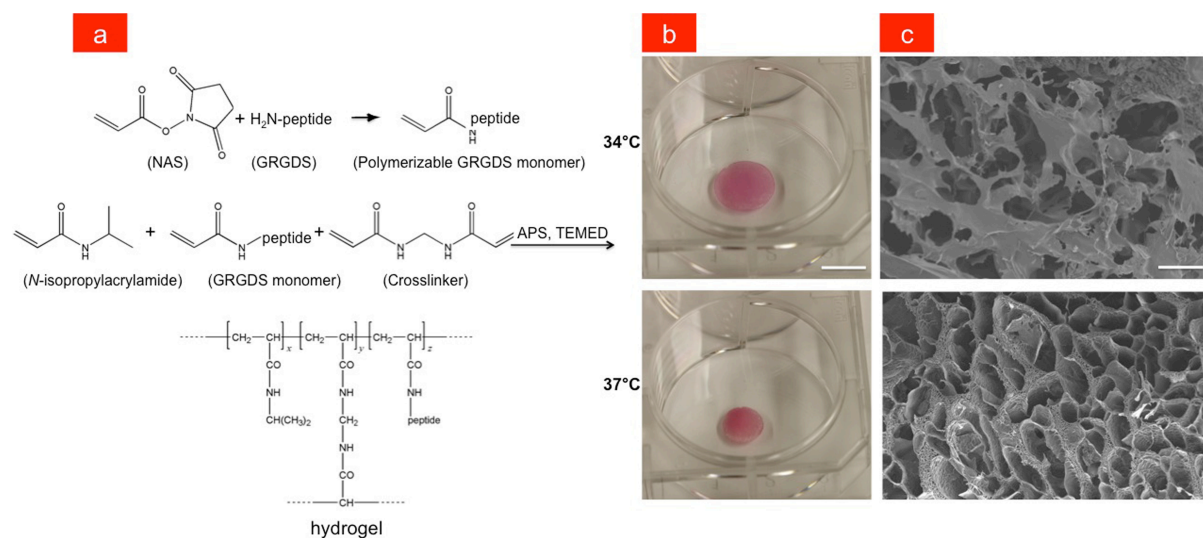


Figure 1. (a) Chemical synthesis of GRGDS-PNIPAAm. First the GRGDS peptide is modified with an acrylate moiety by reaction with *N*-acryloxysuccinimide (NAS) and is then mixed with a NIPAAm and BIS precursor solution. The hydrogel is then polymerized through radical initiation using APS and TEMED. (b) GRGDS-PNIPAAm in swollen and contracted states at 34°C and 37°C, respectively (bar, 1 cm). The red color arises from the hydrogel being submerged in serum-containing medium. (c) SEMs of GRGDS-PNIPAAm gels in swollen (top) and contracted (bottom state showing reduction in pore size with gel contraction (bar, 100  $\mu m$ )).



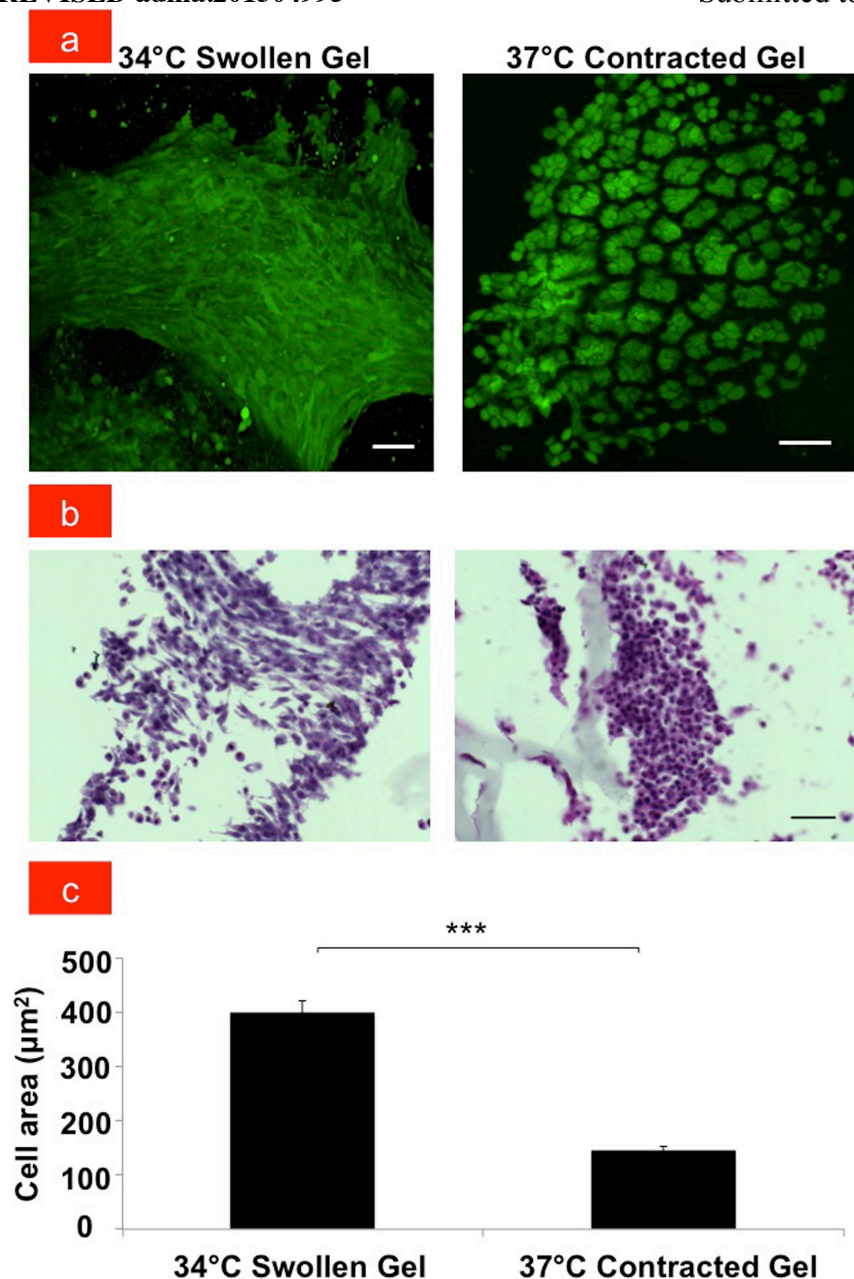


Figure 2. (a) Fluorescent micrographs of E10 dental mesenchymal cells in swollen GRGDS-PNIPAAm hydrogel at 34° C and in contracted GRGDS-PNIPAAm hydrogel at 37° C (bar, 50 μ m). (b) Light micrographs showing hematoxylin and eosin (H&E) staining of mesenchymal cells that appear spread in the swollen GRGDS-PNIPAAm hydrogel, where as they are compact and rounded in the contracted GRGDS-PNIPAAm hydrogel at 37°C (bar, 50 μ m). (c) Graph showing the quantification of the corresponding projected cell areas;

\*\*\* $p < 0.001$ .

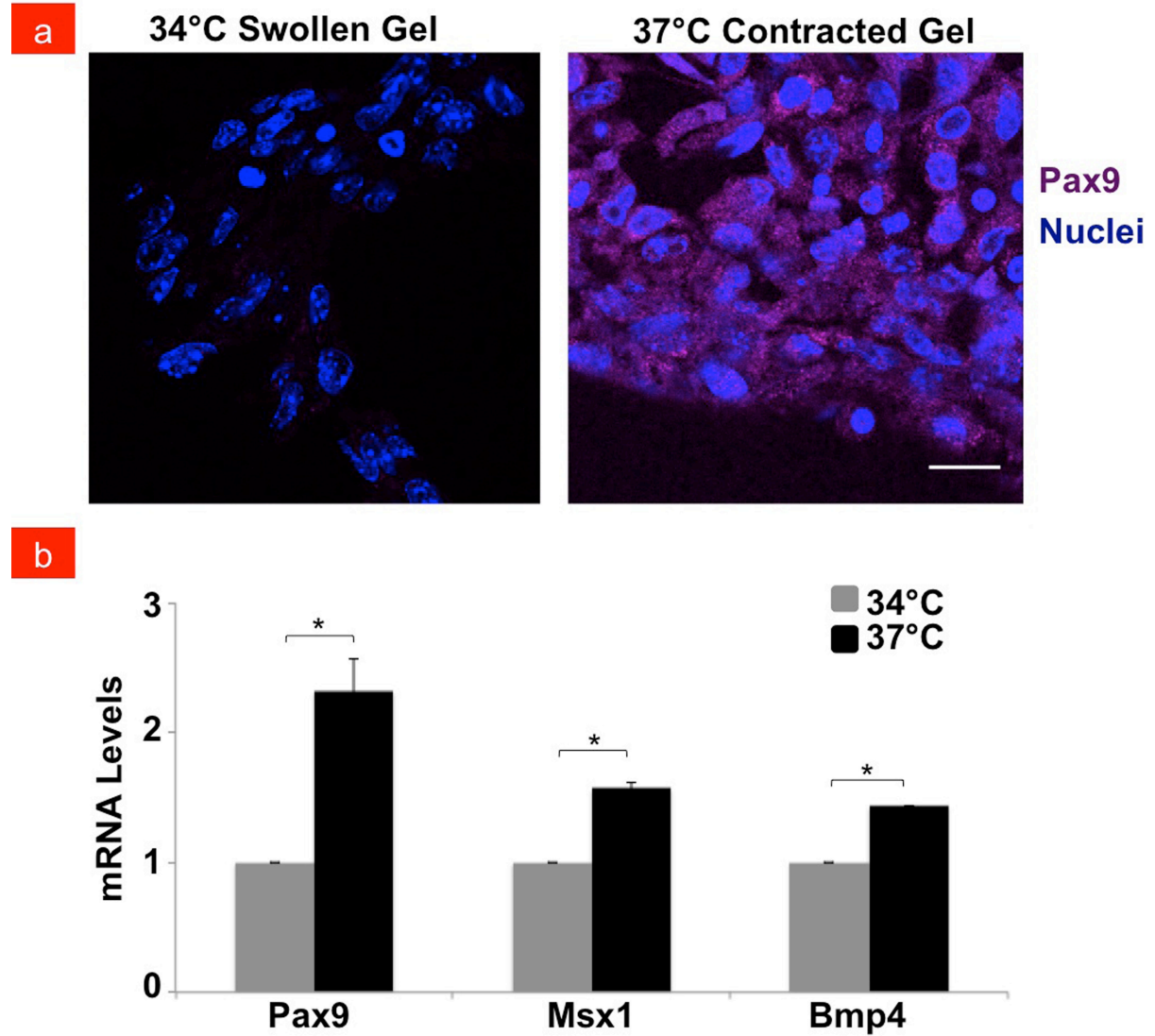


Figure 3. Fluorescent micrographs showing (a) Pax9 protein expression (purple) in mesenchymal cells with their nuclei stained with DAPI (blue) cultured in swollen versus contracted gels at 34°C or 37°C, respectively (bar, 20  $\mu$  m). Pax9 expression is noted in contracted gels and absent in swollen gels. (b) Graph showing the quantification of mRNA expression levels of Pax9, Msx1 and Bmp4 odontogenic markers in compacted mesenchymal cells within contracted PNIPAAm gels. mRNA levels in cells cultured at 37°C (black bars) are shown relative to 34°C controls (grey bars); \* $p$ <0.05.

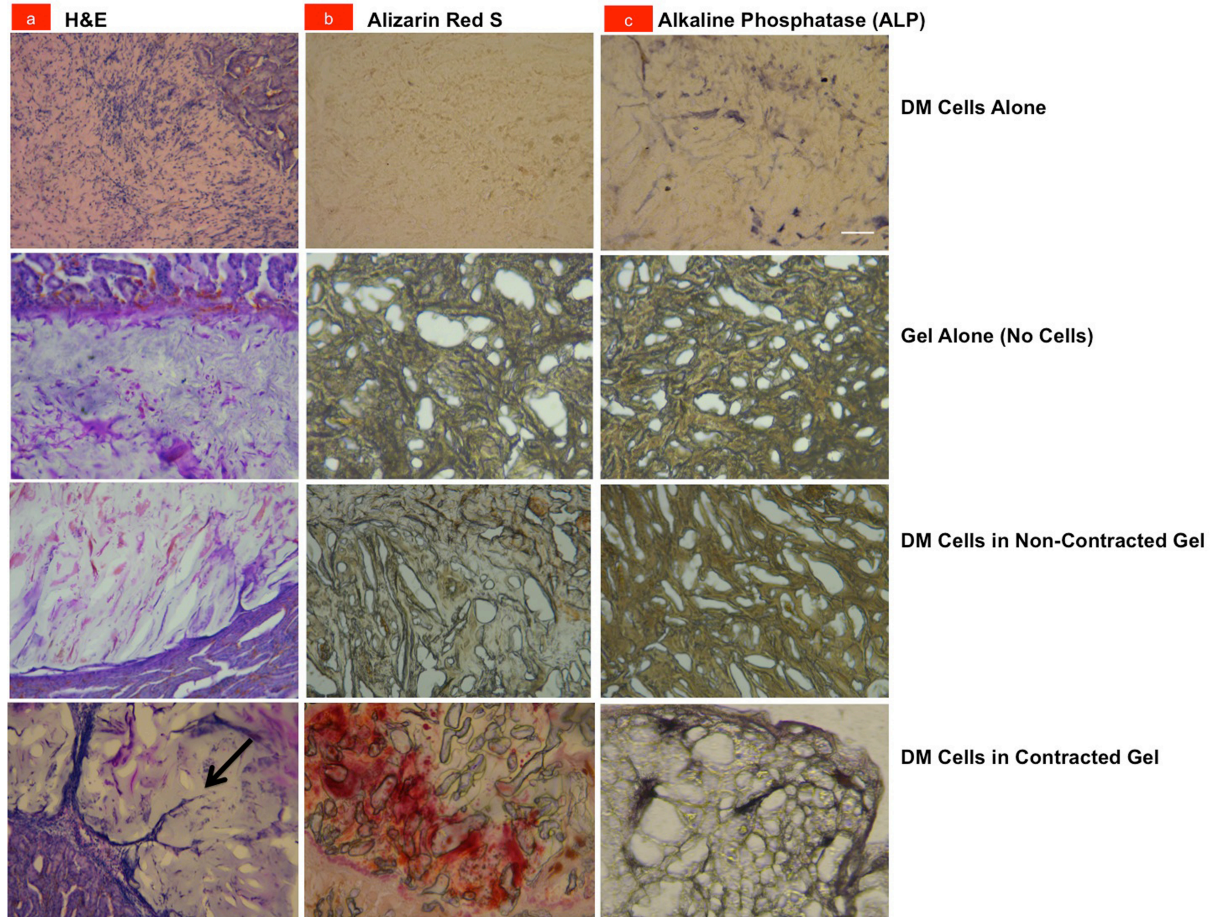


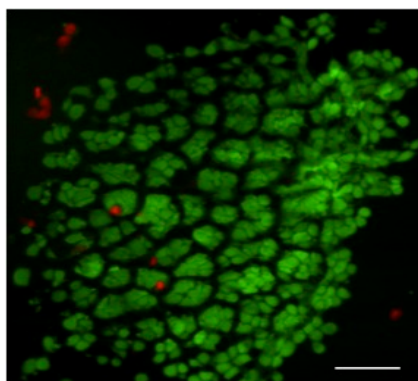
Figure 4. Light micrographs of histological sections of the control dental mesenchymal (DM) cell pellet alone (DM Cells Alone), GRGDS-PNIPAAm gel without cells (Gel Alone/No Cells), DM cells in a non-contracted gel with a LCST>37°C (DM Cells in Non-Contracted Gel), and a contracted GRGDS-PNIPAAm gel containing DM cells (DM Cells in Contracted Gel) when implanted for 2 weeks under the kidney capsule of a mouse. Sections were stained with (a) Hematoxylin and eosin (H&E) or (b) Alizarin Red S, or analyzed for (c) Alkaline Phosphatase (ALP) activity; arrow indicates a new capillary sprout (bar, 100  $\mu$ m).

**TABLE OF CONTENTS ENTRY****Developmentally-Inspired Shrink-Wrap Polymers for Mechanical Induction of Tissue Differentiation**

**A biologically inspired thermoresponsive polymer** has been developed that mechanically induces tooth differentiation *in vitro* and *in vivo* by promoting mesenchymal cell compaction as seen in each pore of the scaffold. This normally occurs during the physiological mesenchymal condensation response that triggers tooth formation in the embryo.

Keywords: scaffold engineering, biomaterials, odontogenesis, poly(*N*-isopropylacrylamide), thermoresponsive

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**Supporting Information**

for *Adv. Mater.*, DOI: 10.1002/adma. (adma.201304995)

**Developmentally-Inspired Shrink-Wrap Polymers for Mechanical Induction of Tissue Differentiation**

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*Experimental Methods*

*Experimental System:* Our methods for separation of dental mesenchyme from dental epithelium of embryonic mouse mandible have been published [1]. In brief, embryos were removed from timed pregnant CD1 mice at E10 and the molar tooth germ was microdissected free under a dissecting microscope. To separate the dental mesenchyme from the epithelium, the tissues were incubated with Dispase (2.4U/ml) with DNase in  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$  free PBS for 20-30 min at 37°C [1]. The tissues were then washed in 10% FBS DMEM, and the epithelium was mechanically pulled free from the dental mesenchyme using fine forceps. Cells from remaining E10 dental mesenchyme were cultured in 10% FBS DMEM in T-75 tissue culture flasks, with medium changed every 2-3 days; all studies were carried with dental mesenchymal cells that were cultured for less than 12 passages.

*Hydrogel Fabrication:* *N*-acryloxysuccinimide (NAS), *N*-isopropylacrylamide (NIPAAm), and *N,N'*-methylenebisacrylamide (BIS) were purchased from Sigma Aldrich; ammonium persulfate (APS) was purchased from Mallinckrodt; tetramethylethylenediamine (TEMED) was purchased from Calbiochem, sodium bicarbonate was purchased from Sigma Aldrich, and GRGDS peptide was purchased from Bachem Biosciences. All were used as received except for the NIPAAm, which was recrystallized from hexane.

The lyophilized acrylate-modified GRGDS was redistributed in hydrogel precursor (10% NIPAAm, 1% BIS w/v in deionized water). The concentration used was determined experimentally to produce gels with a desired LCST  $\approx 36^\circ\text{C}$ . To make a hydrogel scaffold, the

peptide/gel precursor (50  $\mu$ L) was placed in the (1 cm) diameter well of a MatTek glass

bottom dish, then aqueous APS solution (4  $\mu$ L, 10% w/v) was added, followed by TEMED (0.5  $\mu$ L) and stirring. Gelation occurred within a minute. Gels were transferred to a water bath where they were left for 3 days at 4°C to allow any unreacted monomers and initiators to diffuse out of the gel. Each hydrogel was then transferred individually to a centrifuge tube (15 mL) filled partially with water, frozen (-80°C), and lyophilized. Hydrogel characterization was carried out using differential scanning calorimetry (TA Instruments DSC Q200) and the LCST was determined to be  $\approx$ 36°C. For fabrication of gels with a LCST greater than 37°C, the same protocol was followed, except that the concentration of GRGDS peptide was doubled. Scans were performed at a rate of (5°C/min). For scanning electron microscopy (SEM), dry samples were sputter coated with Au/Pd prior to imaging on a JEOL JSM 6390LV SEM.

*Peptide Modification:* GRGDS was modified with an acrylate moiety on the N-terminus to allow copolymerization into the hydrogel scaffolds. GRGDS (6.25 mg) was dissolved in (6 mL) sodium bicarbonate buffer (pH=8.2) and stirred in a round bottom flask while a solution of NAS (0.02g NAS in 3 mL of sodium bicarbonate buffer) was added dropwise. The solution was stirred in the dark for 2.5 hours at room temperature. The solution was dialyzed for 3 days at 4°C against deionized water with frequent bath changes to remove unreacted NAS (Spectra/Por Float-A-Lyzer G2, 10 mL, 0.1-0.5 kD MWCO cellulose ester membrane) and the product was lyophilized.

*Cell Seeding:* Upon polymerization, the GRGDS-PNIPAAm gels were sterilized by being washed with 70% Ethanol-PBS, PBS, and then subsequently submerged in 10% FBS-DMEM at 25°C. Dental mesenchymal cells were then trypsinized and injected in the hydrogel using a (25 gauge) syringe needle at room temperature. The hydrogels with the injected cells were placed overnight in a 34°C incubator under 5% CO<sub>2</sub> to allow for cell adhesion to occur. These hydrogels were then subsequently shifted to a 37°C incubator under 5% CO<sub>2</sub> to induce

their contraction. They were maintained under these conditions overnight for *in vitro*

quantitative PCR assays and a period of 1-3 weeks for cell viability assays and fluorescence imaging.

*Functional Assays:* Cells in PNIPAAm hydrogels were stained using the Live/Dead

Viability/Cytotoxicity Kit for mammalian cells (Invitrogen) according to protocols provided.

For Alkaline Phosphatase (ALP) activity and Alizarin Red S staining, cryosectioned samples on slides were rinsed with calcium and magnesium negative Phosphate Buffer Solution (PBS, Gibco Life Technologies) three times. Samples were then covered in 5-Bromo-4-chloro-3-indolyl Phosphate/Nitroblue Tetrazolium (BCIP/NBT) Liquid Substrate (0.692 mM/L BCIP; 0.734 mM/L NBT) (MP Biomedicals LLC) solution for ALP staining, or in Alizarin Red S powder (20 mg, Sigma Aldrich) mixed with deionized water (20 mL) and adjusted to (pH 6.3). Samples were then rinsed with PBS three times again prior to subsequent mounting and imaging

*Quantitative PCR:* Quantitative PCR was performed to analyze changes in expression levels of key odontogenic regulatory molecules, as previously described [1]. Pax9 antibodies also were purchased from Abcam (Cambridge MA) and corresponding secondary antibody from Invitrogen (Life Technologies).

*Sectioning, Analyses, Imaging:* Our methods for cryosectioning, histological analysis, hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), confocal microscopy, fluorescent microscopy, computerized image analysis have been previously published [1-4].

Anti-Ki-67 antibody was purchased from Abcam (Cambridge MA) and corresponding secondary antibody from Invitrogen (Life Technologies). In brief, an inverted laser scanning confocal microscope (Leica SP5XMP, Buffalo Grove, IL, USA) with acquisition of multiple z-stack sections as well as an inverted fluorescent microscope (Zeiss Axio Observer Z12) with z-stack sections and color camera microscope (Zeiss Axio Zoom V16) were used for imaging and aquisition of time-lapse videos. ImageJ software (NIH Bethesda MD) was used for cell

was used to 3D render the confocal microscope time-lapse and cell volumetric images over time.

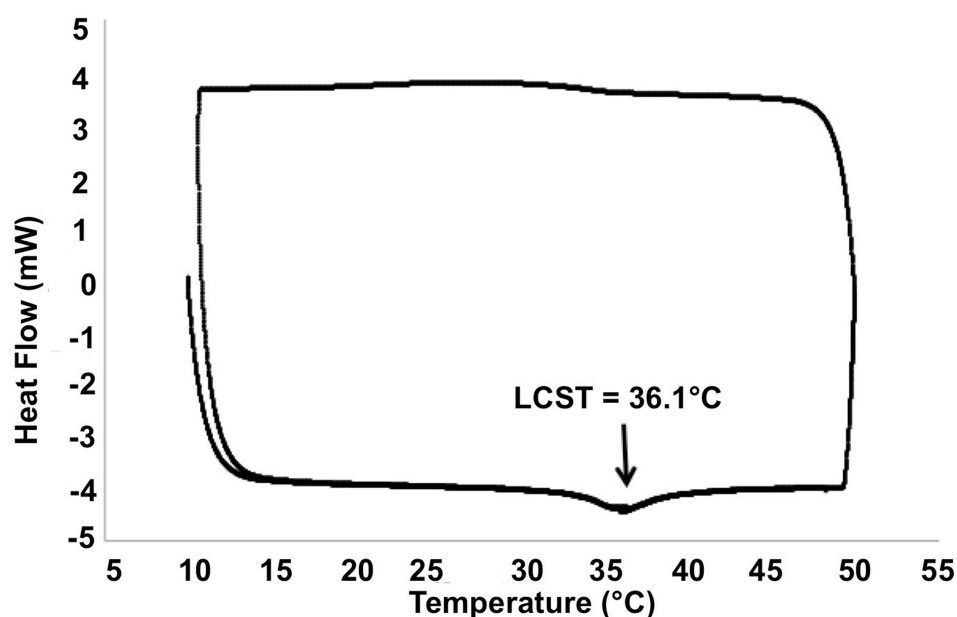
*Animal Experiments:* All animal studies were approved by the Animal Care and Use Committee of Children's Hospital Boston. For *in vivo* experiments, gels placed in a 34°C incubator under 5% CO<sub>2</sub> overnight were implanted under the kidney capsule [5,6] and histological analyses were performed 14 days after the implantation, as previously described [1].

*Statistical Analyses:* Students' t-test was used for cell size (projected cell area) comparison and qPCR results. Results are presented in mean +/- the standard error of the mean unless otherwise stated.

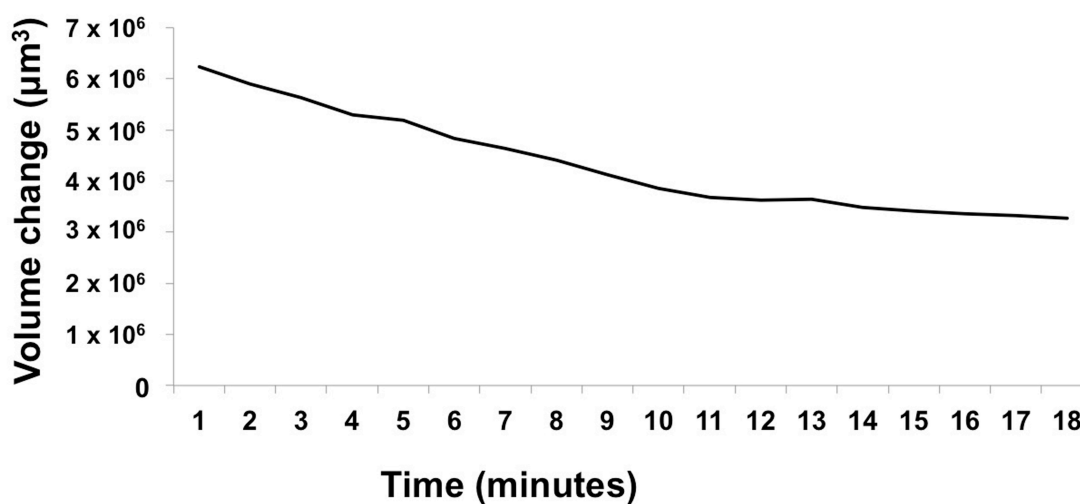
## SUPPLEMENTARY REFERENCES

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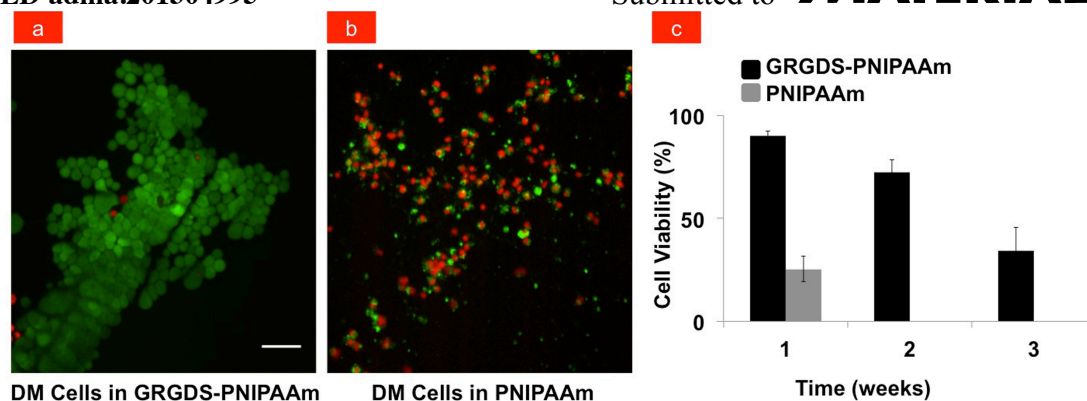


**SUPPLEMENTARY FIGURE LEGENDS**

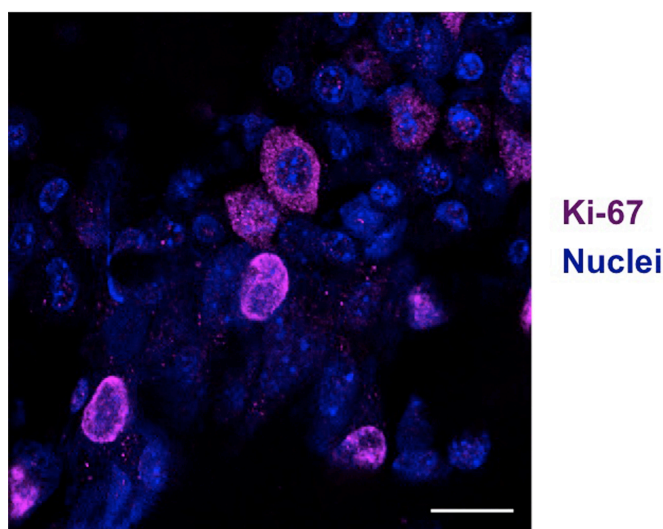
**Figure S1.** Graph of a Differential Scanning Calorimetry plot for a representative GRGDS-PNIPAAm gel indicating the LCST of this material to be  $\sim 36^\circ\text{C}$ .



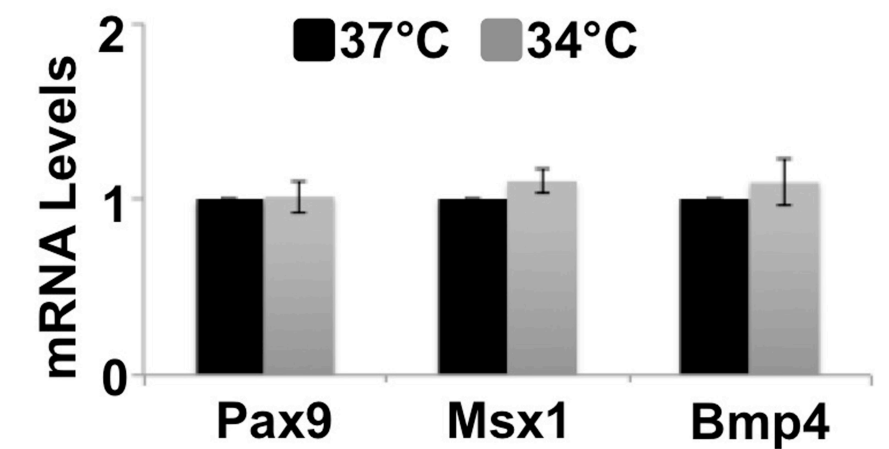
**Figure S2.** Dental mesenchymal (DM) cell volume change in a GRGDS-PNIPAAm gel when induced to contract by heating from  $\sim 30$ - $40^\circ\text{C}$ . Gel contraction initiated soon after the temperature was raised above the  $36^\circ\text{C}$  transition temperature and it reached completion within 10-15 min, as shown in Supplementary Video 1.



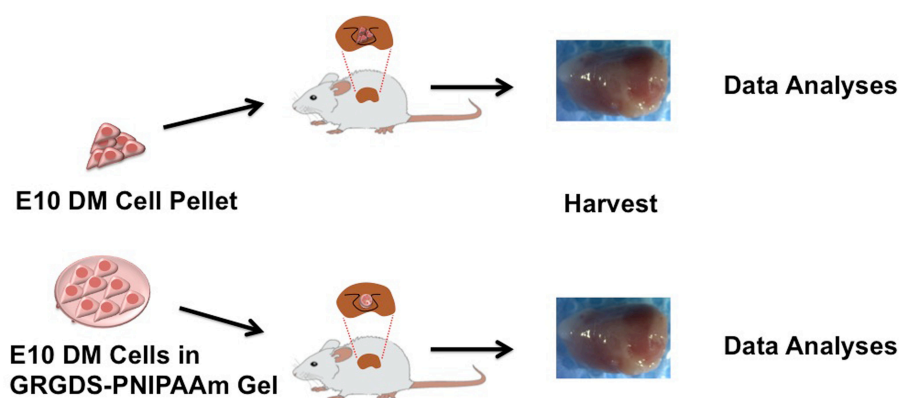
**Figure S3.** Biocompatibility of GRGDS-PNIPAAm gels. (a) Live (green) and dead dental mesenchymal (DM) cells (red) in a contracted GRGDS-PNIPAAm hydrogel after one week. (b) Live (green) and dead cells (red) in PNIPAAm hydrogel of the same formulation but without GRGDS peptide after one week (scale bar, 50  $\mu$ m). (c) Graph showing quantification of cell viability in GRGDS-PNIPAAm hydrogels (black bars) and PNIPAAm hydrogels (grey bars); mean  $\pm$  SEM.



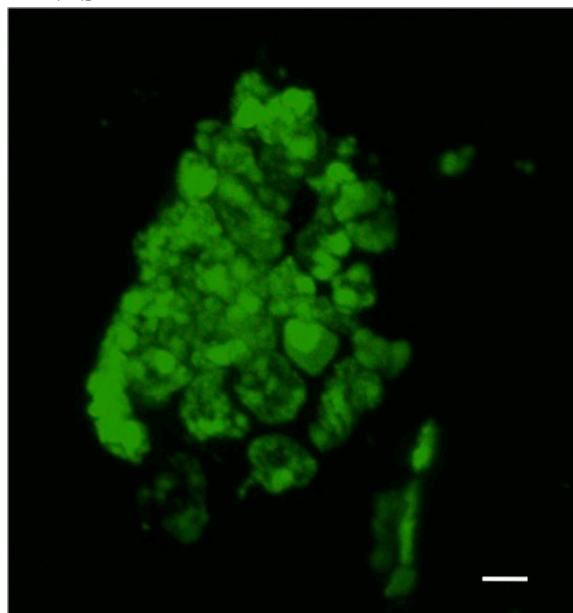
**Figure S4.** Fluorescent micrographs showing Ki-67 protein expression (magenta) in DAPI-labeled nuclei (blue) of dental mesenchymal (DM) cells cultured in a contracted GRGDS-PNIPAAm gels for 3 days (bar, 20  $\mu$ m).



**Figure S5.** mRNA expression of Pax9, Msx1, and Bmp4 in dental mesenchymal cells cultured in 37°C (black bars) and 34°C (grey bars) for 76 hours indicating that temperature difference alone does not significantly influence expression.



**Figure S6.** Representative diagram of experimental protocol for the *in vivo* studies.



**Figure S7.** Compacted GFP-labelled DM cells seeded in a contracting GRGDS-PNIPAAm gel implanted 2 weeks *in vivo* (bar, 20  $\mu$ m).

**Video 1:** A 2D time-lapse recording showing the GRGDS-PNIPAAm gel response to temperature change (from  $\sim 30^{\circ}\text{C}$ - $40^{\circ}\text{C}$ ). The gel rapidly begins to contract once the chamber temperature reaches the gel's LCST of  $\sim 36^{\circ}\text{C}$ , and it completes this response within about 15 min.

**Video 2:** 3D time-lapse recording of GFP-labelled DM cells (green) within the same gel from Video 1 in response to the same temperature change. Once the chamber temperature reaches the gel's LCST, the DM cells immediately compact due to the shrinkage of the gel pores, as shown in Figure 1c.